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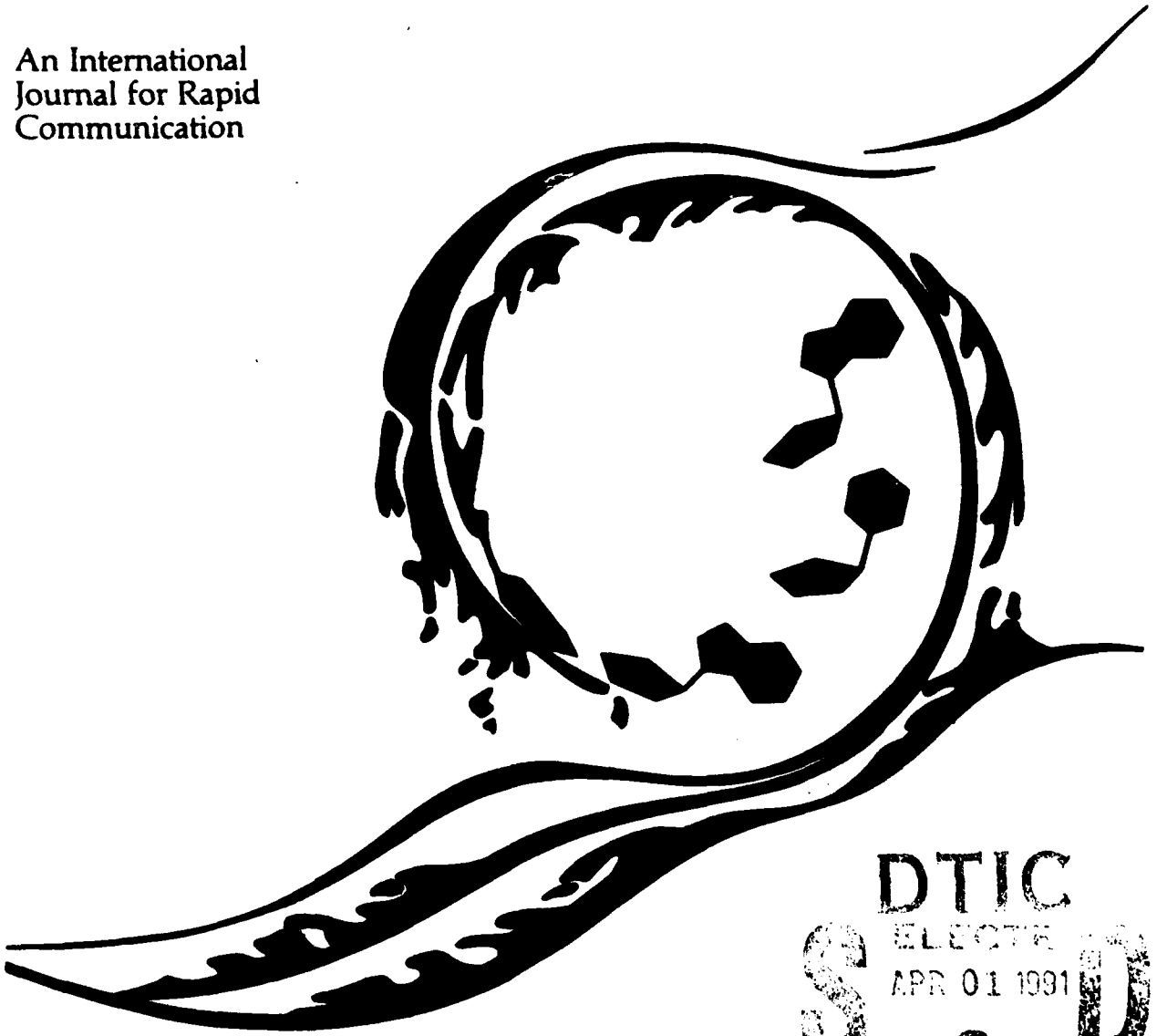

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PREPARATION AND ANTIVIRAL ACTIVITY OF SEVERAL DEOXYGENATED RIBAVIRIN AND TIAZOFURIN DERIVATIVES.

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Abstract: Ribavirin and tiazofurin, two nucleosides of known antiviral activity, have been transformed by previously reported methods to yield several deoxy,epoxy, or dideoxy analogues. The deoxygenated derivatives were evaluated for antiviral activity against a host of DNA and RNA viruses; however, no significant *in vitro* activity was detected.

In the past, a number of 2'3'-dideoxynucleosides have been prepared and evaluated for their antiviral activity.. Such studies were mostly directed towards suppressing the replication of the human immunodeficiency virus in the treatment of the acquired immune deficiency syndrome (AIDS). 3'-Azido-3'deoxy-thymidine (AZT)[1] and 2'3'-dideoxycytidine (ddCyd)[2] were found to be the most active pyrimidine nucleosides, while recent studies indicate that 2'3'-dideoxyinosine (DDI), a purine riboside derivative, might find wide clinical application in the treatment of AIDS.[3]

Since none of the parent nucleosides such as thymidine, cytidine, or inosine show any noticeable antiviral activity, it was thought that the transformation of ribonucleosides of known antiviral activity such as ribavirin and tiazofurin[5] into their deoxygenated derivatives would offer the possibility of augmenting their respective biological activities, or enhancing their therapeutic specificity. Analogously, a recent publication by Krawczyk and Townsend[6] reports the synthesis of the 2'3'-dideoxy derivatives of the antibiotics tubercidin, toyocamycin and sangivamycin as examples of biologically active purine nucleosides which were transformed into agents that might demonstrate anti-HIV activity.

Since the preparation of 2',3'-dideoxyribosides as well as those of other sugar-modified nucleosides has been the topic of a number of studies in recent years, there are several methodologies, such as the modified Corey-Winter reaction[7] and other elimination[8] or synthesis methods[9] available to accomplish such transformations. During the course of this study we found that a modified procedure, based on work reported by Robins et al.[10] was best suited for

transforming both the N-nucleoside ribavirin and the C-nucleoside tiazofurin into various sugar-modified analogues via a common intermediate (3a-d and 9a-d) by using essentially identical reagents and reaction conditions.

Both ribavirin (1) and tiazofurin (9) were acylated with α-acetoxy-isobutyryl bromide (2), as shown in Schemes 1 and 2, to form a mixture of four possible intermediates, shown by structures 3a-d and 9a-d. This mixture of intermediate isomers was subjected to transformations without further characterization; however, upon careful dehydrohalogenation and purification of either 3a-d or 9a-d without deblocking the 5'-position, the ^{1}H NMR spectrum of the purified product 3a or 9a showed the two α-methyl groups of the side chains as one singlet (6H), indicating the existence of the open chain, and not the sterically rigid dioxolone ring configuration as a possible structure.

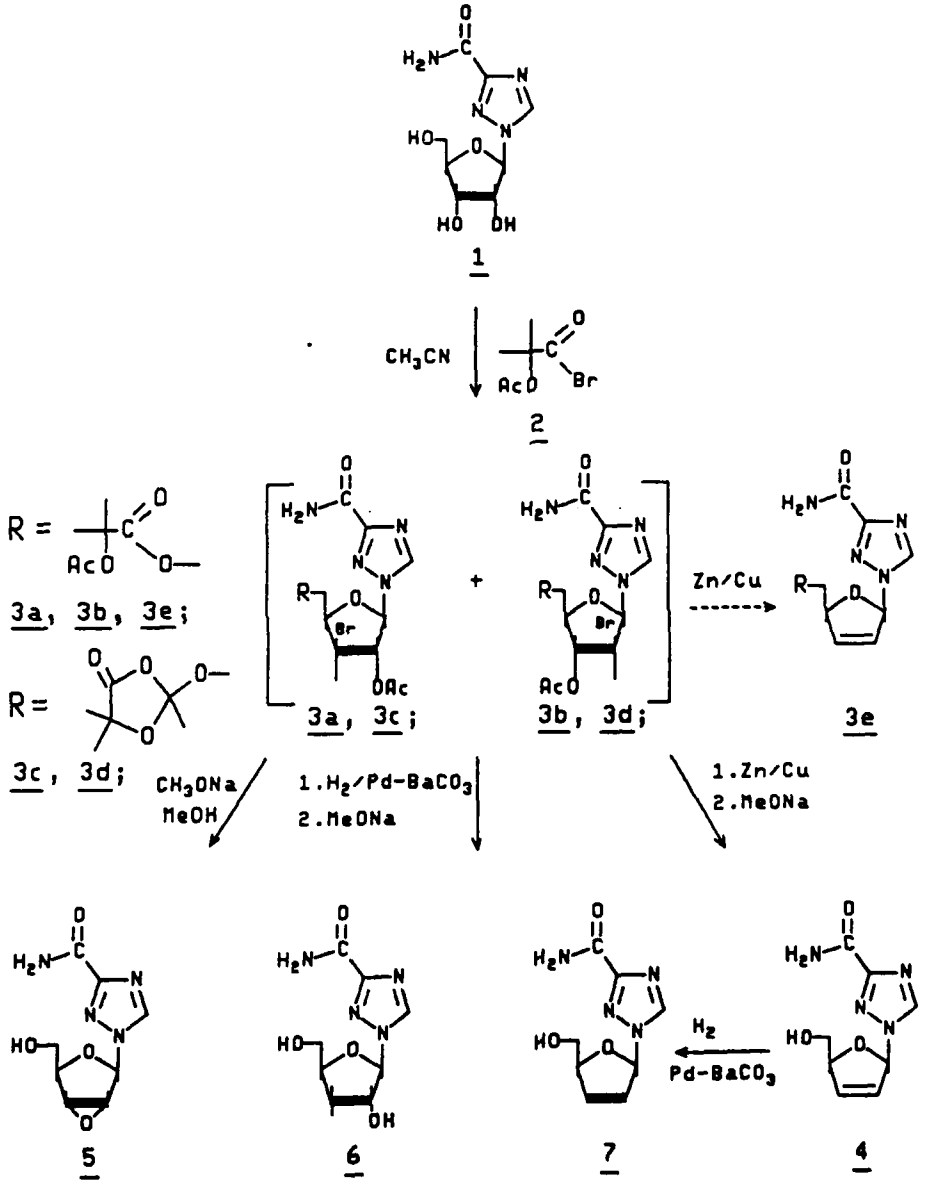
The treatment of 3 and 9 with zinc/copper couple and sodium methoxide readily yielded *enes* 4 and 10 respectively, which in turn were readily hydrogenated to form 2'3'-dideoxyribavirin (7) and 2'3'-dideoxytiazofurin (11) in good yield.

Hydrogenation of 3, followed by deblocking, gave 2'3'-dideoyxribavirin (7). The major product isolated from this reaction, however, was 3'-deoxyribavirin (6), as identified by comparison with data published by Witkowski et al. The treatment of 3 with sodium methoxide in methanol produced 2',3'-anhydroribavirin 5; yet, when the same reaction conditions were applied to 9, it resulted in double elimination and formation of the furan derivative of the thiazole amide 12, first reported by Srivastava et al.[5]

2'3'-Dideoxyribavirin, previously prepared by a different route and shown to be inactive against the HIV virus[12] was still considered a viable candidate to be screened as part of the whole series of obtained compounds against a number of different RNA and DNA viruses, as discussed below.

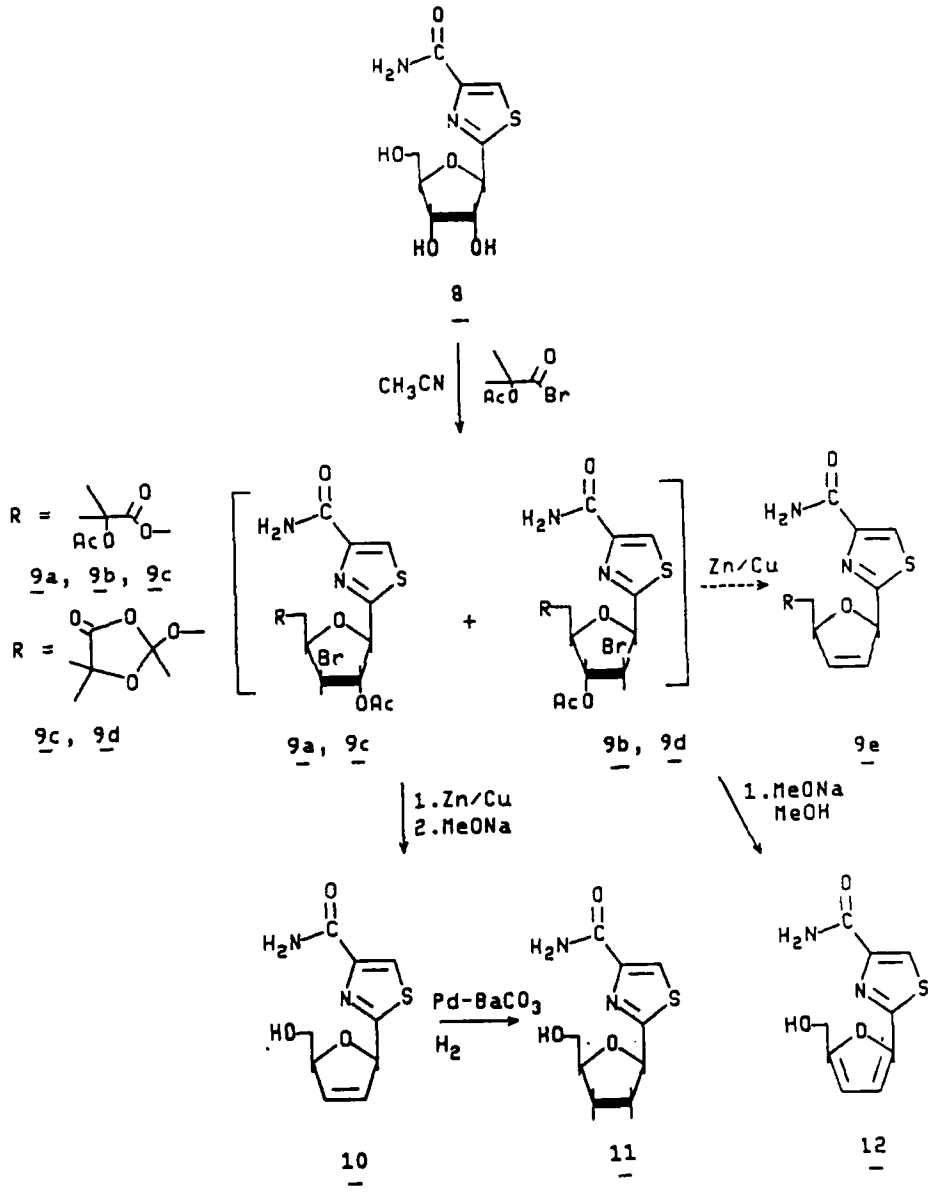
Ribavirin (1) possesses considerable activity in vitro against RNA viruses of the Bunyaviridae family[13,14] (Rift Valley fever, RVF'sandfly fever, SFS, and Punta Toro, PT viruses).[15] Activity has also been demonstrated against the retrovirus human immunodeficiency virus type 1 (HIV-1)[16], the DNA-containing adenovirus type 2 (AD2)[13], and vaccinia virus (VV),[13] and the RNA-containing alphavirus, Venezuelan equine encephalomyelitis virus (VEE)[13,14]. Activity is also present, but to a lesser degree, against RNA viruses of the Flaviviridae family, yellow fever (YF), and Japanese encephalitis (JE) viruses[13,14]. Virtually no activity is observed against vesicular stomatitis virus, VSV (Rhabdoviridae family)[13]. Tiazofurin 8, possesses some activity in vitro against the flaviviruses YF and JE[13,14], lesser activity against the bunyaviruses RVF, PT[13,14] and SFS, and the DNA-containing adenovirus and vaccinia virus[13]. No activity has been reported against HIV, VEE, and VSV[13].

In vitro antiviral activities were determined for the deoxygenated ribavirin analogues 4-7 and tiazofurin analogues 10-12 against human immunodeficiency virus (HIV-1), the RNA-containing bunyaviruses (Rift Valley



Ribavirin Series

SCHEME 1



Tiazofurin Series

SCHEME 2

fever, sandfly fever, and Punta Toro viruses), flaviviruses (Japanese encephalitis and yellow fever viruses), alphavirus (Venezuelan equine encephalomyelitis virus), rhabdovirus (vesicular stomatitis virus), and the DNA-containing adenovirus type 2 and vaccinia virus. The observed antiviral activities are summarized in the accompanying table. Replacement of the ribofuranosyl group in the deoxygenated tiazofurin analogues 10-12 resulted in the loss of all in vitro antiviral activity previously observed for tiazofurin 8 against the flaviviruses, bunyaviruses and DNA viruses, and vaccinia and adenovirus type 2. Compounds 10-12 were also inactive against HIV-1, VEE, and VSV.

IN VITRO ANTIVIRAL TESTING DATA[e]

Compound	Virus	ID_{50}[a]	MTC[b]	TI[c]	[d]TI*
4	RVF	61	<100	1.6	6.6
4	SFS	5	10	2.0	5.6
4	PT	28	32	1.1	6.3
4	YF	73	10	0.1	1.2
4	VV	36	100	2.8	7.5
5	RVF	149	250	1.7	6.6
6	RVF	117	250	2.1	6.6
7	RVF	101	<250	2.5	6.6

[a] 50% Viral inhibitory dose, µg/ml
[b] Minimum Toxic Concentration, µg/ml
[c] Therapeutic Index, TI = MTC_{50}/ID_{50}
[d] Positive drug controls : ribavirin (RVF, SFS, PT), selenazofurin (YF, JE), adenosine arabinoside, ara-A (VV)
[e] Vero cells

Replacement of the ribofuranosyl group of ribavirin 1 by 2',3'-dideoxy (7), 3'-deoxy (6), or 2',3'-anhydro (5) ribofuranosyl moieties resulted in elimination of all antiviral efficacy against HIV-1, vaccinia and adenoviruses, flaviviruses (JE, YF), Venezuelan equine encephalomyelitis (VEE), bunyaviruses (PT, SFS) and no resulting activity against vesicular stomatitis virus (VSV). 2',3'-Dideoxy-2',3'-didehydro ribavirin 4 retained some efficacy only against the bunyaviruses (RVF, PT, SFS) and vaccinia virus, however the level of efficacy in vitro was greatly reduced compared to that of ribavirin. Similar reduced activity was also observed against Rift Valley fever virus by 5-7. Plaque reductions of 80% (@ 100 ug/mL), 59%, 76% and 94% were observed for 4-7 respectively against RVF virus in Vero cells at 250 ug/mL. However the activities of 4-7 against RVF could not be separated from the accompanying Vero cell toxicity. 2',3'-Dideoxyribavirin 7 and 3'-deoxyribavirin 6 were evaluated further in the murine model of Rift Valley fever virus[17]. Doses of 25, 125 and 250 mg/kg/day were administered subcutaneously in 10% DMSO-PBS or saline on days

-1 to +3. No beneficial effects were observed in terms of increased survival numbers or times, nor were the compounds toxic at these doses (virus ratings, VR, 0.96-0.99). As a positive control, ribivirin at doses of 100 and 200 mg/kg/day protected 100 % of the RVF-infected mice (VR = 5.4 and 6.1 respectively).

EXPERIMENTAL SECTION

Analytically pure ribavirin and tiazofurin were provided by US Army Medical Research Institute for Infectious Diseases, Ft. Detrick, MD.

Melting points were determined on a Thomas Hoover melting point apparatus and are uncorrected. The utilized Zn/Cu couple contained 5% copper. Silica gel used for chromatography was flash grade (Aldrich, 260-400 mesh), and thin-layer chromatography (TLC) was performed on prescored silica gel plates GHLF, 250 microns (Analtech Corp., Newark, DE.) with 6:1 dichloromethane-methanol as developing solvent. TLC plates were sprayed with 10% methanolic sulfuric acid after elution and heated to visualize the compounds. IR spectra were recorded using a Beckman AccuLab 2 spectrophotometer, and elemental analyses were performed by MHW Laboratories, Phoenix, Arizona.

All of the NMR spectra with the exception of 12 were obtained on a Varian VXR500S NMR spectrometer equipped with a SUN 4/110 acquisition computer and data station. The following 90° pulse widths were used for 1D and 2D data acquisition: proton, observe = 14.0 μsec, Waltz decouple = 89.3 μsec; carbon, observe = 15.0 μsec, Waltz decouple = 30.8 μsec, 2D 90° PW = 29 μsec. For 1D experiments, the Ernst angle was used for acquisition. Heteronuclear multiple quantum coherence (hmqc) standard pulse sequence from Varian software was used to obtain directly bonded, indirectly detected proton-carbon connectivities (ref. Bax, $^1J_{CH}$ = 150 Hz)[18]. Heteronuclear multiple bond connectivity (hmbc) was modified from Varian software according to Bax[18] and used to detect long range proton-carbon connectivities (Jnxh - 8 Hz). Standard Varian COSY was used for proton-proton connectivity determination. Zero field decoupling (ZFD) and modified Varian software were used to obtain chemical shift and coupling information.

Spectra of 12 were obtained on a Varian XL200 with an ADVANCE data system operating at 200.1 MHz. The following 90° pulse widths were operational: proton,observe = 23.5 μsec, Waltz decoupling = 79.2 μsec; carbon, observe = 9 μsec. The Ernst angle was used for 1D data acquisition.

Definition of J coupling notations: capital letters in ^{13}C coupling patterns refer to directly bonded $^1J_{C-H}$ while lower case letters refer to J coupling over more than one bond. For example, Ddt (156, 2.0, 10.8) means that the carbon in question has a directly bonded J_{CH} of 156 Hz, a long-range coupling to one proton of J = 2.0 Hz and a long range coupling to two protons of J = 10.8

Hz, s = singlet, d = doublet, t = triplet, q = quartet, b = broad, cm = complex multiplet.

The chemical shifts in the proton spectra are referenced from tetramethylsilane (TMS) set equal to 0 ppm. The chemical shifts in the carbon spectra are referenced with respect to dimethylsulfoxide-d_6 ($DMSOd_6$) set equal to 39.5 ppm from TMS.

In vitro antiviral activity was determined in terms of therapeutic index by observing inhibition of viral cytopathic effect (CPE)[13,15,19-22] except for RVF virus which was determined by plaque reduction assays [14]. The 50% inhibitory dose is that drug dose causing a 50% inhibition of CPE or plaque number. The minimum cytotoxic concentration (MTC) is that drug concentration at which 50% of the cells showed cytotoxic effects. The in vitro therapeutic index (TI, proportional to in vitro activity) was calculated by dividing the MTC by the ID50. Compounds were evaluated for therapeutic efficacy in Rift Valley fever-infected mice according to the procedure of Peters et al[17].The in vivo virus rating, VR, was calculated by dividing the geometric mean time to death of drug-treated, infected animals by that for untreated, infected animals.

1-(2,3-Dideoxy-ß-D-glycero-pent-2-enofuranosyl)-1,2,4-triazole-3-carboxamide (4). (2',3'-Dideoxy-2',3'-didehydroribavirin):

Ribavirin (1) (19.5 g, 80 mmol) was dissolved in acetonitrile (200 mL) containing water (1.44 mL, 80 mmol). To this solution was added α-acetoxyisobutyryl bromide (2) (49.4 g, 36 mL, 240 mmol) in one portion, and stirring was continued at room temperature for two hours. After adding 5% sodium bicarbonate solution (200 mL) the mixture was extracted with ethyl acetate (2 x 200 mL), and the organic phase was washed with sodium bicarbonate solution and with brine. After evaporation of the solvent under reduced pressure a highly viscous foam was obtained, which was dissolved in tetrahydrofuran (600 mL). Zinc/copper couple (80 g) was added, followed by ammonium chloride (50 g) and the reaction mixture was stirred for two hours when the temperature reaches 40°. The zinc/copper couple was filtered off, washed with ethyl acetate and the organic layer was washed with a 5% aqueous solution of ethylenediamine tetraacetic acid tri-sodium salt, followed by washings with bicarbonate (100 mL) and brine (200 mL).

The solvent was removed under reduced pressure, the residue was dissolved in methanol (200 mL) and sodium methoxide (0.5 g) was added to adjust the pH to 9.5. After stirring for three hours a solid started to precipitate. The solvent volume was reduced to half its volume, the precipitate was collected by filtration and recrystallized from methanol-ethyl acetate.

Yield 7.0 g (42%); m.p. 152-153°; IR (KBr): 3400-3050; 1750; 1480; 1750; 1480; 1270; 1190; 1070; 840; 780 cm^{-1}. ^{1}H-NMR: (DMSO-d_6) δ 8.75(s, 1, C_5H); 7.82 and 7.63 (each singlets, 1H each, NH); 6.85(td,1,H-1',J(1',2') = 1.6 Hz, J(1',4') = 2.4 Hz); 6.51 (td, 1,^{6}H-3',J(3',4') = 1.7 Hz, J(3',2')=6.1 Hz); 6.13 (ddd,

1,[a1]H-2',J(2',1') = 1.5 Hz, J(2',4') = 2.3 Hz, J(2',3') = 6.0 Hz); 4.914 (ddddd, 1, H-4', J(4',1') = 2.4 Hz, J(4',3') = 1.7 Hz, J(4',2') = 2.3 Hz, J(4,5'a,b) = 4.2, 4.8 Hz, 4.908 (4, 1, 5'-OH, J(OH-5' = 5.6 Hz; 3.48, 3.55 (AB of ABXY, 2, H-5'a,b,J(gem) = 11.6, 11.7, J(5',a,b,4')=4.2, 4.8Hz, J(4', OH) = 5.6 Hz);

^{13}C-NMR: (DMSO-d^6): δ 160.32 (Sq, C=O, J = 1.2 Hz); 156.75 (Sdd, C-3, J = 8.5, 11.7 Hz); 144.13 (Dd, C-5, J = 214.7, 2.7 Hz); 134.58 (Dtdd, C-3', J = 172.3, 4.0, 2.4, 7.4 Hz); 124.56 (Dtd, C-2', J = 176.9, 4.1, 2.5 Hz); 93.24 (Dtd, C-1', J = 171.1, 10.6, 3.6 Hz); 88.61 (Dddt, C-4', J = 149.8, 8.8, 11.3, 2.2 Hz); 62.84 (T, C-5', J = 141.0 Hz);

TLC: R_f 0.7. Anal. Calcd. for $C_8H_{10}N_4O_3$: C, 45.61; H, 4.79; N, 26.66;. Found: C, 45.85; H, 4.76; N, 26.85.

1-(2,3-Dideoxy-ß-D-glycero-pentofuranosyl)-1,2,4-triazole-3-carboxamide (7) (2',3'-Dideoxyribavirin):

Ribavirin-2'-ene (4) (2.3 g, 11 mmol) was dissolved in methanol (100 mL), and palladium on barium carbonate (500 mg) was added. The mixture was hydrogenated at room temperature and atmospheric pressure for 3 hours. The catalyst was filtered off on a sintered glass funnel, the filtrate was evaporated to dryness under reduced pressure, and the residue was recrystallized from methanol (50 mL) to yield 1.8 g (77%) of dideoxyribavirin, m.p. 153-154°.(lit[12] 154°C) IR (nujol): 3000-2800 (br); 1690; 1460; 1370 cm^{-1}.

^{1}H NMR:[12] (DMSO d_6) 12: δ 8.81 (s, 1, C_5H); 7.79, 7.59 (each singlet, 1, NH); 6.16 (dd, 1, H-1', J(1'-2'a,b) = 2.6, 6.5 Hz); 4.88 (t, 1, 5'-OH, J = 5.6 Hz); 4.15 (dddd, 1, H-4', J = 5.2, 4.5, 6.0, 9.2 Hz); 3.56 (ddd, 1, H-5'a, J = 11.7, 4.2, 5.7 Hz); 3.47 (dt, 1, H-5'b, J = 11.7, 5.3 Hz); 2.38 (cm, 2, H-2'a,b); 1.98 (cm, 2, H-3'a,b).

^{13}C-NMR: (DMSO d_6): δ 160.37 (S, C=O); 156.84 (Sdd, C-3, J = 8.2, 11.4 Hz); 143.88 (Dd, C-5, J = 214.2, 1.8 Hz); 88.61 (Dcm, C-1', J_{ch} = 170.1 Hz); 82.84 (Dcm, C-4', J_{ch} = 146.3 Hz); 62.86 (Td, C-5', J = 139.8, 4.7 Hz); 31.90 (Tt, C-3', J = 134.1, 3.1 Hz); 25.32 (Tcm, C-2', J_{ch} = 133.0 Hz).

TLC: Rf 0.65. Anal. Calcd. for $C_8H_{12}N_4O_8$: C, 45.27; H, 5.70; N, 26.40. Found: C, 45.26; H, 5.72; N, 26.36.

3'-Deoxyribavirin (6):

Ribavirin (1) (4.88 g, 20 mmol) was dissolved in acetonitrile (60 mL) and α-acetoxyisobutyryl bromide (2) (9 mL, 50 mmol) was introduced in one portion. The reaction mixture was stirred for two hours at room temperature, then ethyl acetate (300 mL) was added to the clear solution. The organic layer was washed with 5% sodium bicarbonate solution (2 x 50 mL), the bicarbonate phase was washed with ethyl acetate (100 mL), and the combined organic phase was washed with water

[1]Assigned from coupled ^{13}C spectrum through HMQC.

(2 x 50 mL) and saturated brine (50 mL). The ethyl acetate solution was dried over sodium sulfate, and the solvent was evaporated under reduced pressure to yield 9.2 g of (3) as a viscous oil.

The crude material was dissolved in dry methanol (200 mL), then triethylamine (3 mL) was added, followed by 5% palladium on barium carbonate (2 g). The reaction mixture was hydrogenated at room temperature and atmospheric pressure for two hours, then stirring was continued for four more hours. The catalyst was filtered off, the solvent was removed under reduced pressure and the residue was vacuum-dried. After dissolving the residue in methanol (200 mL) sodium methoxide (1.5 g) was added, and after two hours TLC indicated the completion of deblocking, showing the presence of two products: the spot at R_f 0.7 indicated dideoxy-didehydro-ribavirin (4) while the major product at R_f 0.3 represented 3'-deoxy-ribavirin (6).

The solvent was evaporated under reduced pressure, the residue was loaded onto a silica gel column and eluted with methylene chloride, gradually increasing its polarity by adding methanol. Collecting the fractions containing the two compounds, 0.5 g of dideoxy-didehydro-ribavirin (4) and 2.1 g (47%) of 3'-deoxy-ribavirin (6) was obtained, m.p. 141-142°.(lit[11] 141-142°).

IR (nujol): 3400-3000 (br); 2950; 1680; 1600; 1455; 1300; 1110; 1080; 710 cm^{-1}.

^{1}H NMR (DMSO-d_6): δ 8.87 (d, 1, C_5H, $^4J(5,1') = 0.2$ Hz); 7.85 (bs, 1, NH); 7.64 (bs, 1, NH); 5.86 (d, 1, H-1', $J(1'-C_5H) = 0.7$ Hz); 5.74 (bd, 1, 2'-OH, $J = 3.8$ Hz); 4.98 (bs, 1, 5'-OH); 4.47 (cm, 1, H-4'); 4.43 (bdt, 1, H-2', $J(2'-OH) = 5$ Hz, $J(2',3') = 9.8$ Hz); 3.65 and 3.52 (both dd, 1 each, H-$5'_{a,b}$, $J(5'^a,5'_b) = 11.7$ Hz, $J(5',4') = 2.4, 4.5$ Hz); 2.12 and 1.90 (both ddd, 1 each, H-$3'_{a,b}$, $J(gem) = 13.2$ Hz, $J(3',2') = 10.1$ Hz, $J(3',4') = 1.5, 5.1$ Hz.

^{13}C-NMR (DMSO-d_6): δ 160.59 (cm, C=O); 157.26 (Sdd, C-3, $^3J = 8.5, 11.6$ Hz); 144.27 (Dd, C-5, $J = 215.6, 2.0$ Hz); 94.67 (Dcm, C-1', $^1J_{CH} = 170.3$ Hz); 82.37 (cm, C-41, $1J_{CH} = 148.9$ H_3); 75.50 (Dcm, C-2', $J_{CH} = 153.2$ Hz); 62.59 (Td, C-5', $J = 140.2, 3.7$ Hz); 33.77 (Tcm, C-3', $J_{CH} = 132.3$ Hz).

TLC: Rf 0.3. Anal. Calcd. for $C_8H_{12}N_4O_4$: C, 42.10; H, 5.30; N, 24.55. Found: C, 42.22; H, 5.41; N, 24.35.

1-(2',3'-Anhydro-ß-*D*-ribofuranosyl)-1,2,4-triazole-3-carboxamide (5)

Ribavirin (1) (2.44 g, 10 mmol) was dissolved in acetonitrile (30 mL) containing water (0.18 mL). While stirring α-acetoxyisobutyryl bromide (2) (4.5 mL, 30 mmol) was added in one portion. After 2 h at room temperature ethyl acetate (200 mL) was added, the solution was washed with sodium bicarbonate solution 5% (2 x 50 mL), the bicarbonate solution was extracted with ethyl acetate (100 mL) and the combined ethyl acetate extracts were washed with water (2 x 50 mL) and saturated brine solution.

The organic phase was dried over sodium sulfate, filtered, and, upon evaporation of the solvent, 5 g of crude material was obtained. The crude

product (5 g) was dissolved in 1 M methanolic sodium methoxide solution (40 mL) and stirred for two hours, during which time a solid separated from solution. The solid was collected by filtration and recrystallized from water to yield 1.8 g (80%) of final product, m.p. 233-235° IR (nujol): 3430; 3265; 3000-2800 (br); 1690; 1600; 1460; 1375; 1290; 1190; 1070; 1030; 970; 860; 830 cm^{-1}.

^{1}H NMR (DMSO-d_5): δ 8.347 (s, 1, C_5H, ^4J < 0.4 Hz if present); 7.879 (s, 1, NH); 7.705 (s, 1, NH); 6.281 (s, 1, H-1', J < 0.7 Hz if present); 4.985 (t, 1, 5'-OH, J = 5.5 Hz); 4.30 (dd, 1, H-2', ^3J(2',1') = 0.5 Hz, J(2',3') = 2.7 Hz); 4.21 (d, 1, H-3', J (3',2') = 2.7 Hz, coupling was small to H-4' if present); 4.18 (dd, 1, H-4', J(4',$5'_{a,b}$) = 5.8, 6.8 Hz); 3.63 (ddd, 1, H-$5'_a$) and 3.47 (ddd, 1, H-$5'_b$), J($5'_a$,$5'_b$) = 11.4, 11.3 Hz, J($5'_{a,b}$, OH) = 5.6, 5.7 Hz, J($5'_{a,b}$,4') = 6.8, 5.6 Hz.

^{13}C-NMR (DMSO-d^6): δ 160.11 (Sdd, C=O, ^2J(C-N-H) = 1.1, 2.2 Hz; 157.45 (Sdd, C-3, ^3J = 8.8, 11.5 Hz); 145.56 (Dd, C-5, J = 215.7, 1.6 Hz); 85.26 (Ddd, C-1', J = 171.6, 5.9, 10.6 Hz); 81.21 (Ddcm, C-4', J = 151.9, 11.7, complex multiplet); 60.52 (Tcm, C-5', J = 142.1, complex multiplet); 57.43 (D of pentuplets (to H-$5'_{a,b}$, H-4' and H-2') C-3', J -193.4, 4.6 Hz); 57.16 (Dt, C-2', J = 197.8, 4.0 Hz).

TLC: Rf 0.32. Anal. Calcd. for $C_8H_{10}N_4O_4$: C, 42.47; H, 4.45; N, 24.77. Found: C, 42.46; H, 4.51; N, 24.88.

2-(2,3-Dideoxy-ß-*D*-glycero-pent-2-enofuranosyl)-thiazole-4-carboxamide (10) (2',3'-dideoxy-2',3'-didehydrotiazofurin):

Tiazofurin (8) (5.12 g, 20 mmol) was suspended in acetonitrile (60 mL) containing water (0.36 mL), and α-acetoxyisobutyryl bromide (2) (9 mL, 60 mmol) was added in one portion. After stirring at room temperature for three hours ethyl acetate (200 mL) was added, and the solution was washed with 5% sodium bicarbonate (2 x 50 mL). The aqueous layer was extracted with ethyl acetate (100 mL), the combined organic layers was washed with water (2 x 50 mL) and brine (50 mL), followed by drying over sodium sulfate.

The solvent was evaporated under reduced pressure and the thus obtained foam was dissolved in tetrahydrofuran (200 mL). Zinc-copper couple (25 g) and ammonium chloride (12 g) were added and the mixture, initially at 40°C, was stirred while allowing the temperature to adjust to room temperature. After 2.5 hours, the Zn/Cu-couple was filtered off, the solvent was evaporated under reduced pressure, and the residue was taken up in ethyl acetate (300 mL). The solution was washed with a 5% EDTA tri-sodium salt solution (2 x 50 mL), the aqueous phase was extracted with ethyl acetate (100 mL) and the combined organic layer was washed with water (100 mL) and brine (50 mL). After drying over sodium sulfate the solvent was evaporated under reduced pressure, the residue was dissolved in methanol (100 mL), and sodium methoxide (0.5 g) was added to a pH of 10. After stirring for two hours TLC indicated complete disappearance of starting material and Amberlite H^+ resin was added to neutralize the medium.

The resin was filtered off, and the solvent was evaporated under diminished pressure. The residue was chromatographed on a silica gel column with dichloromethane/5% methanol as eluant. Removal of solvent in vacuo from fractions containing 10, followed by recrystallization from ethyl acetate gave 3.9 g (86%) of 10, m.p. 120-121°. IR (nujol): 3460; 3300-3000(br); 2950; 2840; 1645; 1570; 1450; 1360; 1280; 1070; 1030 cm^{-1}.

^{1}H NMR: (DMSO d_6) δ 8.2 (d, 1, $C_5\underline{H}$, J(5-1') = 0.4 Hz); 7.7 and 7.6 (each bs, 1 each, NH, slight exchange with D_2O); 6.17 and 6.13 [(AB of ABXY, 2, H-3' and H-2' respectively;[a] J(2',3') = 6.1 Hz, J(2', 1') = 1.8 Hz, J(2',4') = 2.1 Hz, J(3',4') = 1.4 Hz, J(3',1') = 2.3 Hz]; 6.02 (dddd, 1, H-1', J = 0.4, 1.6, 2.1, 3.8 Hz; 4.93 (t, 1, 5'-OH, exchanged with D_2O, J = 5.6 Hz); 4.92 (dddt, 1, H-4', J = 1.5, 2.3, 3.8, 5.4 Hz, couplings to H-3', H-2', H-1' and H-5'a,b respectively); 3.59 and 3.53, (AB of ABXY, 2, H-5'a,b; J(gem) = 11.2 Hz, J(5'-4') = 5.4, 5.4 Hz, J(5'-OH) = 5.7, 5.4 Hz.

^{13}C-NMR: (DMSO d_6) δ 173.1 (Stdd, C-2, J = 1.7, 5.2, 7.2 Hz)[a2]; 162.3 (Sd, C-4, $^2J_{CH}$ = 1.7 Hz); 150.2 (Sdd, C=O, J = 4.5, 6.8 Hz); 130.3 (Dsextets, [b3]C-2', J = 171.6, 3.6 Hz); 128.6 (Dq, [b]C-3', J = 175.0, 4.2 Hz); 124.7 (D, C-5, $^1J_{CH}$ = 192.7 Hz); 88.5 (Dtq, C-4', J = 148.3, 10.0, 2.4 Hz); 84.5 (Dt, C-1', J = 153.8, 10.7 Hz); 64.4 (Tdd, C-5', J = 141, ca. 3, ca. 7 Hz); [a] Two $^3J_{CH}$ to H-5, H-2'; 1.7 Hz coupling to H-1' or H-3'.

TLC: R_f 0.7. Anal. Calcd. for $C_9H_{10}N_2O_3S$: C, 47.77; H, 4.45; N, 12.38; S, 14.17. Found: C, 47.80; H, 4.62; N, 12.13; S, 13.94.

2-(2,3-Dideoxy-β-D-glycero-pentofuranosyl)thiazole-4-carboxamide (11) (2',3'-dideoxytiazofurin): Tiazofurin-2'-ene (2.5 g, 10 mmol) was dissolved in methanol (100 mL), and maintained under a nitrogen atmosphere. Carefully 5% ethanol-pretreated palladium on barium carbonate (1 g) was introduced, and the hydrogenation was carried out at room temperature and atmospheric pressure during a two hour period. The catalyst was filtered off, the solvent was evaporated under reduced pressure and the residue was recrystallized from ethyl acetate; yield 2.1 g (84%); m.p. 94-95°. Analysis showed that the compound crystallized with 0.5 mol of water. IR (KBr): 3400-3050; 1670; 1380; 1190; 1050; 940 cm^{-1}.

^{1}H NMR: (DMSO d_6) δ 8.81 (s, 1, $C_5\underline{H}$); 7.79 and 7.59 (each bs, 1 each, NH, partially exchanged with D_2O); 5.20 (dd, 1, H-1', J(1'-2'a,b) = 5.4, 7.9 Hz)[a4]; 4.88 (t, 1, 5'-OH, partially exchanged with D_2O, J = 5.5 Hz); 4.08 (tdd, 1, H-4', J(4'-5'a,b) = 5.3 Hz, J(4'-3'a,b) = 6.1, 7.5 Hz); 3.54 and 3.49[a] (each dd,

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[2]Definitive assignment from ^{13}C spectrum based on hmbc present for H-5'a,b: δ 6.17, but not for δ 6.13.

[3]Assigned from hmbc.

[4]In a D_2O-exchanged sample, J=0.9; 4.6 Hz.

1 each, H-5'a,b, J(5'a,b-4') = 5.4, 5.2 Hz, J(gem) = 11.3 Hz); 2.41 (cm, 1, H-2'a); 2.03 (cm, 2, H-2'b, H-3'b); 1.71 (cm, 1, H-3'a).

[a5]Irradiation of H-4' produced no change in the absorption of H-1', indicating the absence of H(1'-4') coupling through the ribosyl oxygen or through C_2-C_3. The latter was observed when C_2-C_3 was unsaturated. Irradiation of H-4' gave rise to an AB pattern for H-5'a,b.

^{13}C-NMR: (DMSO d_6) 175.30 (Std, C-2, J = 4.5, 7.2 Hz); 162.80 (S, C-4); 150.15 (Sddd, C=O, J = 0.8, 4.9, 6.8 Hz[b6]); 124.38 (D, C-5, J_{CH} = 192.3 Hz); 81.73 (Dcm, C-4', J = 147.4, 8.2 Hz); 78.12 (Dcm, C-1', J = 151.8, 7.3 Hz); 63.93 (Tdd, C-5', J = 139.6, 2.0, 4.5 Hz); 33.09 (Tcm, C-3', J = 133.4 Hz); 27.71 (Tcm, C-2', J = 129.2 Hz);

TLC: Rf 0.70. Anal. Calcd. for $C_9H_{12}N_2O_3S$.: C, 47.35; H, 5.30; N, 12.27; S, 14.04. Found: C, 47.16; H, 5.41; N, 12.13; S, 13.78.

2-(5-Hydroxymethylfuran-2'-yl)thiazole-4-carboxamide (12):

Tiazofurin (1) (2.6 g, 10 mmol) was suspended in acetonitrile (30 mL) containing water (0.18 mL, 10 mmol) and α-acetoxyisobutyryl bromide (2) (4.5 mL, 30 mmol) is added in one portion. The reaction mixture was stirred for two hours when it formed a clear solution. Ethyl acetate (200 mL) was added, and the solution was washed with 5% sodium bicarbonate solution (2 x 50 mL), the aqueous phase was extracted with ethyl acetate (100 mL x 2), and the combined organic extract was washed with water (50 mL) and brine (50 mL). After drying over sodium sulfate the solvent was evaporated to yield a crude reaction product mixture (9).

The crude product was dissolved in anhydrous methanol (100 mL) and sodium methoxide (1.5 g) was added to adjust the pH value to 10. After stirring for two hours at room temperature the reaction mixture was neutralized with Amberlite resin H^+ (20 g). The resin was collected by filtration, the solvent was evaporated and the residue was recrystallized from methanol (25 mL) to yield 1.9 g (85%) of pure product, m.p. 192-194°; (lit. 192-193°). IR (KBr): 3420; 3380-3050(br); 1680; 1550; 1380; 1295; 1070; 1020; 890; 810 cm^{-1}.

^{1}H NMR: (DMSO d_6) δ 200 MHz 8.25 (s, 1, C_5H); 7.75 and 7.66 (each bs, 1 each, exchangeable with D_2O, NH); 7.11 (d, 1, [a]H-2', J(2'-3') = 3.4 Hz); 6.55 (d, 1, [a]H-3', J(3'-2') = 3.4 Hz); 5.45 (t, 1, 5'-OH, exchangeable with D_2O, J = 5.6 Hz); 4.50 (d, 2, H-5'a,b, J= 5.35 Hz); [a7]

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[5]Definitive assignment from ^{13}C spectrum based on hmbc present for H-5'a,b: δ 6.17, but not for δ 6.13.

[6]In a D_2O-exchanged sample, J=0.9; 4.6 Hz.

[7]Tentatively assigned. Similar chemical shifts and couplings were reported by Srivastava, et al[5]

^{13}C-NMR: (DMSO d_6) δ (Coupled with D_2O exchange); 162.35 (Sd, C-4, ^{2}JCCH = 1.4 Hz); 157.8 (Scm,[b8] C-4'); 157.2 (Sd, C-1', ^{2}JCCH = 7.7 Hz); 151.0 (Sdd, C=O, ^{3}JCCCH = 4.6 Hz, ^{2}JCNH = 7.2 Hz); 147.0 (Sdd, C-2'[b] ^{3}JCSCJ = ^{3}JCCCH = 8.4 Hz); 123.5 (Ds, C-5'[b] $^1J_{CH}$ = 194.7 Hz[c])[9]; 110.8 (Dd, C-2', J = 178.3, 4.6 Hz); 109.8 (Ddt, C-3', J = 177.2, 2.8, 3.8 Hz); 55.7 (Td, C-5', J = 142.8, 2.9 Hz).

TLC: Rf 0.55. Anal. Calcd. for $C_9H_8N_2O_3S$: C, 48.20; H, 3.60; N, 12.50; S, 14.30. Found: C, 48.38; H, 3.72; N, 12.49; S, 14.16.

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[8]Most highly coupled carbon.

[9]The carbon chemical shifted and assignments for the thiazole ring for compounds 10-12 (Scheme 2) agree generally with those of Kovacs, et al. (23). The sole exception was C-2 of 12 which was shifted upfield to 147 ppm from its usual absorption at 172-3 ppm by the direct bonding to the furanosyl ring.

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